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THE VARIATION IN ISOMETRIC TENSION WITH SARCOMERE LENGTH IN VERTEBRATE MUSCLE FIBRES

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SUMMARY

1. The variation of isometric tetanus tension with sarcomere length in single fibres from frog striated muscle has been re-investigated with special precautions to ensure uniformity of sarcomere length within the part of the fibre being studied.

2. In most respects the results of Ramsey & Street (1940) were confirmed, but (*a*) the peak of the curve was found to consist of a plateau between sarcomere lengths of 2·05 and 2·2 μ, (*b*) the decline of tension above this plateau is steeper than found by Ramsey & Street, and (*c*) the decline of tension below the plateau becomes suddenly steeper at a sarcomere length of about 1·67 μ.

3. Many features of this length–tension relation are simply explained on the sliding-filament theory.

4. It is concluded that, in the plateau and at greater lengths, the tension on each thin filament is made up of equal contributions from each bridge which it overlaps on adjacent thick filaments.

5. Internal resistance to shortening is negligible in this range but becomes progressively more important with shortening below the plateau.

INTRODUCTION

In the preceding paper (Gordon, Huxley & Julian, 1966) we described measurements of tension and stiffness in muscle fibres that were stretched to such an extent that there was no overlap of thick and thin filaments. We found that although there were suggestions that both of these quantities increased slightly on stimulation, the changes were of a smaller order of magnitude than those which occur when there is substantial overlap, and

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we concluded that the relatively large tensions found by other workers in these conditions were chiefly due to non-uniformity of striation spacing, so that overlap, although absent for most of the length of the fibre, was nevertheless present in places, especially near the fibre ends (A. F. Huxley & Peachey, 1961).

The present paper deals mainly with the tension generated by isolated fibres from frog muscle at different degrees of stretch covering the whole range of lengths within which the two sets of filaments overlap. In general the results confirm those of Ramsey & Street (1940) very satisfactorily, but several new features have emerged which correlate closely with expectations based on the filament dimensions given by Page & H. E. Huxley (1963) and by H. E. Huxley (1963). A few experiments on the speed of shortening at different lengths are also reported.

The methods have in the main been described in the preceding paper. A few improvements in the apparatus are described here under 'Methods'.

Preliminary accounts of the results have already been published (A. F. Huxley, 1963; Gordon, Huxley & Julian, 1964; Huxley & Julian, 1964).

METHODS

Most of the experiments were carried out over the same period as those described in the preceding paper (Gordon *et al.* 1966), and the apparatus is described there in detail. The measurements of tension near the optimum length, however, were repeated after the apparatus had been improved in several ways which will be described under the heading 'Modifications to Apparatus'.

Principle of the method

A part of the length of an isolated muscle fibre, within which the striation spacing was sufficiently uniform, was defined by two 'markers' (pieces of gold leaf) stuck to the fibre with tap grease. The length L of the part between these markers was measured continuously by a photo-electric device (the 'photo-electronic spot follower'). L could be held constant ('length control') by feeding from the output of this device to a servo motor which pulled on the tendon at one end of the fibre; stimulation in this condition produced an isometric contraction of the part of the fibre between the markers, and the tension was recorded by a transducer attached to the other tendon. Alternatively, the tension could be held constant ('tension control') by feeding from the tension signal to the servo motor; the contraction was then isotonic and the shortening of the part between the markers was signalled by the photo-electronic spot follower. A diode circuit allowed tension control to take over when tension reached a pre-set level, and length control to take over again when shortening reached a pre-set value, so as to make the part of the fibre between the markers undergo an afterloaded contraction with shortening stop.

Modifications to apparatus

Servo motor. The moving-coil motor described in the previous paper was replaced by a moving-iron torque motor (Elliott, model 130). The principal advantage of this motor was the rigidity of its rotor and bearings, as a result of which the loop gain could be increased until the deflexion in response to a square pulse was about complete in 1 msec. Since it was used only for small length changes the 'slider' was omitted and the tendon was attached to a hook fixed directly to the arm on the motor shaft. The arm was a re-inforced tripod structure built up from dry grass stems fixed together, and coated externally, with a waterproof adhesive (Araldite, Ciba Ltd.), which gave a better combination of internal rigidity and low inertia than light-alloy tubes or several other materials that were tried.

Angular position of the motor shaft was detected by a vane in a capacitance gauge (Cambridge & Haines, 1959). This signal could be used as one of the inputs to the servo system instead of the 'length' signal derived from the photo-electronic spot follower; also this signal was differentiated (time constant 0·1 msec) to provide the velocity signal needed to stabilize the servo. A small amount of positive feed-back from this position signal cancelled out the restoring torque of the motor.

The amplifiers driving the motor were re-designed, and negative feed-back from the current through the motor was provided so as to overcome the inductance of the windings.

The steady-state stiffness of the system was about 10 g wt. per micron of length error, i.e. about 100 times higher than with the original moving-coil motor.

Length measurement. Dial gauges, divided to 0·01 mm and capable of being read to 0·001 mm, were used in place of the verniers for measuring lateral movements of the stage and of the travelling microscope with which the distance between the spots of the spot-follower was measured.

Stimulation. Transverse stimulation was used. Alternating condenser discharges with time constant about 2 msec were passed between platinized platinum plates about 5 mm apart extending along the whole length of the fibre.

Procedure in experiments near optimum length

In both series of experiments, the contractions were afterloaded with the shortening brought to an end at the length at which the isometric tension was to be measured, since straightforward isometric tetani at these lengths are not possible with this apparatus because the fibre is so slack that the servo is not stable. The isotonic phase shows up as a step in the rise of tension in Fig. 1.

Procedure in final series of experiments. Except during the contractions, the motor was fed from the position signal derived from the capacitance gauge attached to its shaft. The stability of the servo was therefore independent of the properties of the fibre, and a shorter initial length could be used than if the motor was fed from the 'length' signal. Before each contraction, the length of the fibre was adjusted until the separation of the spots on the cathode ray tube of the spot-follower was at the value corresponding to a striation spacing 0·3 μ greater than that at which the tension was to be measured. When, on stimulation, the tension rose above the pre-set level of the afterload, the contraction became isotonic, and it became isometric when the length signal (from the spot follower) reached the pre-set level and came through the diode switching circuit to feed the main amplifier and motor. The stimulus frequency was adjusted in each experiment to the lowest value that gave satisfactory fusion. Tetani were given at regular intervals of either 4 or 5 min. After each tetanus the fibre was stretched and released, passively, four or five times, over a range of striation spacings from about 2·1 to 2·5 μ, with the intention of smoothing out any irregularities of spacing which might have developed during the tetanus (compare Gordon *et al.* 1966, p. 160). Every third tetanus was at a 'standard' length (striation spacing about 2·1 μ). The tension corresponding to the standard length was interpolated between the values measured at this length, and the tension measured at each other length was expressed as a percentage of the relevant interpolated value. For measurement, the records were projected on to millimetre squared paper and the height of the plateau was read to 0·1 mm (0·1–0·2 % of the height). All records were read independently by two of us; their values (percentage of 'standard length' tension) seldom differed by as much as 0·5 %, and the mean was used. One experiment had to be excluded because the output from the tension transducer was fluctuating enough to introduce appreciable errors. The first run in the experiment of 27 October 1964 was excluded because the intervals between tetani were not equal and the tensions at standard length were therefore erratic. The useful part of each experiment was terminated by the appearance of notches in the tension records, presumably due to the occasional failure of propagation of the action potential. All other results from the final series of experiments are included in Table 1. The excluded results are similar but more scattered.

RESULTS

Isometric tension at different sarcomere lengths

For practical reasons, it was not possible to investigate the whole range of lengths on any one fibre. Among the reasons were the following: (1) different placing of the markers was required for different parts of the length range; (2) the neighbourhood of the peak of the curve by itself required a long series of measurements; (3) the measurements at high degrees of stretch had to be made at the beginning of an experiment, in order to have maximum uniformity of striation spacing; (4) it was not desirable to do the experiments at moderate lengths on fibres which had previously been stretched so far that the filaments no longer overlapped, since it was not known whether the filaments would return to a completely normal arrangement. In particular, the fibres used in the experiments described in the preceding paper were not used for any of the observations near the peak of the length–tension curve or at still shorter lengths.

Peak of length–tension curve. A series of experiments carried out in 1963 showed a plateau with practically constant tension over the range 2·0–2·2 μ sarcomere length, and there were fairly sharp corners at the ends of this plateau beyond which the tension declined in each direction. These observations were the basis of our preliminary report (Gordon *et al.* 1964). The differences between this relation on the one hand, and a smooth curve fitted to the points on the other, were however quite small (about 1 %), and we therefore repeated the experiments at the end of 1964 after we had made the improvements to the apparatus and procedure which are described in the Methods section of this paper. Only this second set of experiments is presented here.

Representative records from one run are reproduced in Fig. 1. The length trace (upper) shows the shortening, amounting to 0·3 μ/sarcomere in every record, but it does not show the differences in initial length, since the latter were set in on the 'L_p' control (Gordon *et al.* 1966, Fig. 5), and the trace shows deviations from the value set.

The results from the whole series are collected in Table 1, and the means

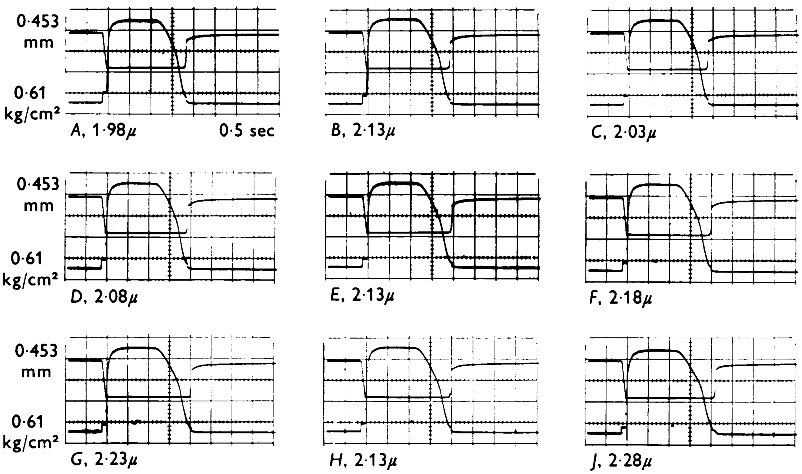

Fig. 1. Tetani at lengths near the optimum for tension development. From run 3 of 8. xii. 64 (see Table 1); 3–4° C. Lower trace, tension; upper trace, changes of length (between markers). Sensitivity equal in all records; values at left-hand side refer to 1 large square of grid. 0·453 mm length change equivalent to 0·18 μ change in striation spacing. Upstroke of tension record retouched in *A* and *B*.

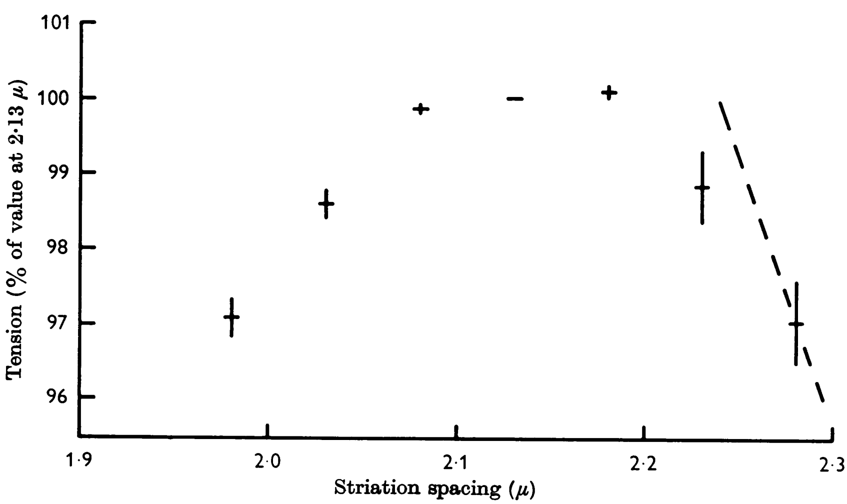

Fig. 2. Tension developed at striation spacings near the optimum. Mean values from Table 1; vertical lines show ± 1 standard error of the mean. Interrupted line on right reaches zero tension at a striation spacing of 3·65 μ.

TABLE 1. Tension developed at lengths near the optimum. Expressed as percentage of that at the 'standard length', 2·13 μ. Temperature 3–4° C in all experiments. Arrows indicate whether the observations were made in the sequence of increasing or decreasing striation spacing. In experiments of 27 October 1964, striation spacings were 0·02 μ smaller than indicated in the column headings; on 1 December 1964 they were 0·01 μ smaller and on 26 November 1964 they were 0·01 μ greater

Date	Run no.	Direction	Maximum tension developed (kg/cm^2)	Stimulus: Duration (sec)	Stimulus: Frequency (sec^{-1})	Tension developed at striation spacing of: 1·98 μ	2·03 μ	2·08 μ	2·13 μ	2·18 μ	2·23 μ	2·28 μ
27. x. 64	2	←	3·4	1·0	15	96·83	99·45	99·85	100	99·85	96·75	95·20
10. xi. 64	1	←	2·7	1·0	20	97·15	98·00	99·95	100	99·95	98·10	96·45
26. xi. 64	1	→	2·2	1·3	15	96·05	99·00	100·20	100	100·25	—	—
1. xii. 64	1	←	2·8	1·0	10	96·48	98·10	99·75	100	100·20	99·63	97·45
8. xii. 64	1	→	2·6	1·5	14	97·55	98·45	99·95	100	100·45	99·75	98·25
8. xii. 64	2	←	2·6	1·5	14	97·65	98·72	99·68	100	99·78	99·63	97·95
8. xii. 64	3	→	2·6	1·5	14	97·95	98·50	99·70	100	100·15	99·25	—
					Mean:	97·09	98·60	99·87	100	100·09	98·85	97·06
					S.E. of mean:	0·26	0·19	0·07	—	0·09	0·49	0·56

are plotted against striation spacing in Fig. 2. The existence of a plateau is clearly confirmed; its limits are at about 2·05 and 2·2 μ striation spacing. It is noticeable that the standard errors are much smaller for the two points adjacent to the 'standard' length than for the points above or below the plateau; this is probably because length errors will have an effect proportional to the slope of the graph. For example, Table 1 shows that in the experiment of 26 November 1964 the tension is below average for the two shortest lengths and above average for the two longest. The amount of these deviations would correspond to an error of about 1% in the determination of striation spacing.

The values shown in our preliminary note (Gordon *et al.* 1964) for the limits of the plateau were 2·0 and 2·2 μ. The difference in the figure for the lower end is very likely due partly to the improvements in technique between the two series of experiments, but it probably also represents a real variability between fibres. The results can best be summarized in an idealized fashion by drawing straight lines meeting the plateau level at 2·25 and approximately 2·0 μ, and rounding the corners so as to leave a true plateau extending between 2·20 at the top, and at the bottom, 2·0–2·05 in different fibres.

Decrease of tension above optimum length. The range of lengths between the upper end of the plateau and the length where tension development failed almost completely was investigated in some of the experiments directed principally at one or other of these two ranges. A difficulty in investigating this range is that the tension records showed an appreciable amount of 'creep' (e.g. Figs. 9 and 11, Gordon *et al.* 1966), similar to but smaller than that described by Ramsey & Street (1940, Fig. 8). There can be little doubt that this is due to the progressive development of irregularities of striation spacing during the tetanus which is to be expected because of the instability represented by the negative slope of this part of the length–tension relation (Hill, 1953). This effect is greatly reduced in our apparatus because the part of the fibre being investigated has initially a striation spacing which is much more nearly uniform than that of a whole fibre, but nevertheless a slight progressive tension rise is to be expected from this cause. Although we have no direct evidence that this is the cause of the 'creep' we do regard it as sufficiently probable to lay more emphasis on a value of tension extrapolated back to the beginning of the tetanus by the procedure indicated in Fig. 3 than on the maximum tension reached.

The results from four experiments are plotted against striation spacing in Fig. 3. It will be seen that the tensions extrapolated in this way fall satisfactorily close to a straight line drawn from maximum tension at a spacing of 2·25 μ to zero at 3·65 μ, these being respectively the 'corner' at the upper limit of the plateau determined in the previous section, and the point where developed tension approaches zero as determined in the preceding paper.

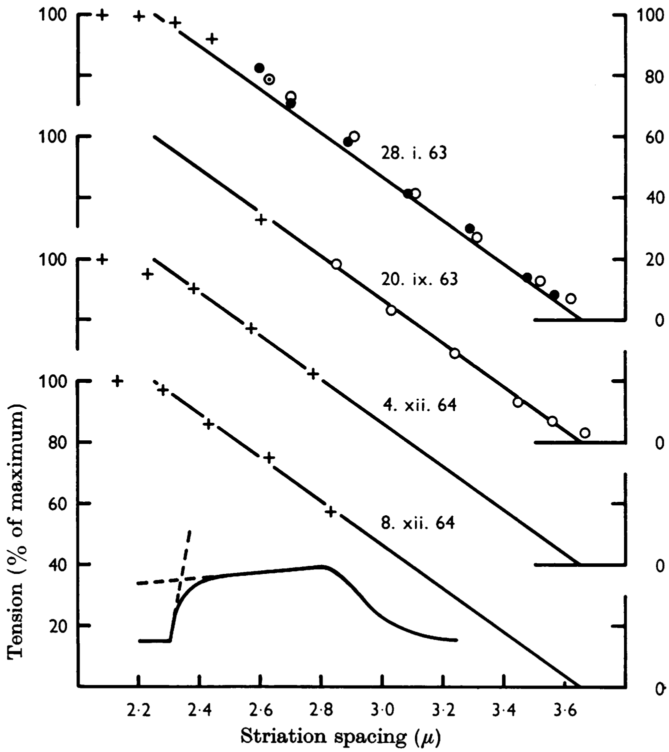

Fig. 3. Tension developed at lengths above the optimum. Extrapolated back to start of tetanus by the procedure shown in the inset. Circles, isometric; crosses, after-loaded contractions with light load and shortening terminated at the striation spacing shown. 28. i. 63: filled circles, decreasing series of lengths; open circles, increasing series immediately afterwards. Scale for these points adjusted to match the point ⊙, which represents an isometric contraction at the end of the isotonic series, later in the experiment. 20. ix. 63: scale arbitrary because no measurement was made near the optimum. Lines drawn from zero at $s = 3{\cdot}65\ \mu$ to 100% at $s = 2{\cdot}25\ \mu$.

Decrease of tension below the optimum length. In three experiments, tension development was recorded at lengths well below the plateau. Sample records are reproduced in Fig. 4 and the tensions are plotted against striation spacing in Fig. 5. The decline of tension becomes steeper at a sarcomere length between 1·65 and 1·70 μ and the tension approaches zero at about 1·3 μ. The tetani were of moderate duration (not exceeding 1·4 sec at 4–5° C), and were not long enough to produce the 'delta state' of Ramsey & Street (1940).

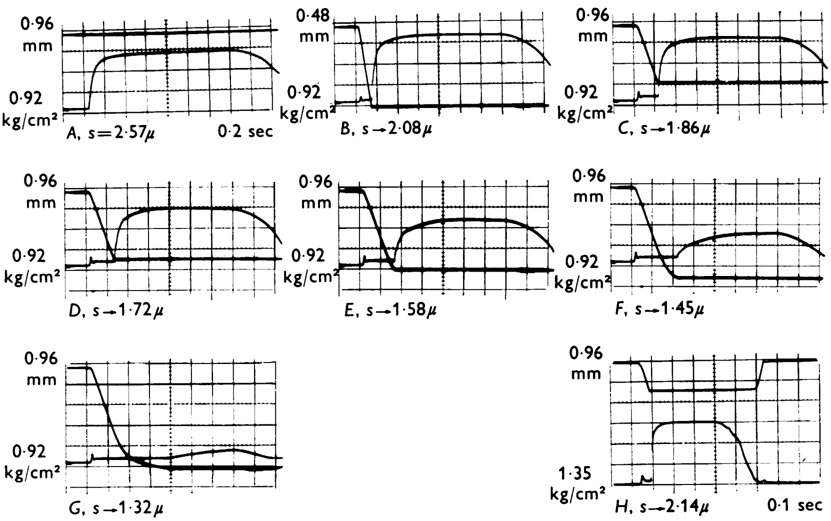

Fig. 4. Afterloaded contractions. Upper trace, distance between markers; lower trace, tension. *A–G*, experiment of 7. xi. 63, 4° C, stimulated at 20/sec. *A*, isometric at striation spacing $s = 2{\cdot}57\ \mu$. *B–G*, initially at $s = 2{\cdot}57\ \mu$, then shortening allowed at load of about 0·2 kg/cm² until shortening stop is reached at the striation spacing indicated under each record. *H*, from another experiment (11. x. 63, 23–24° C) to illustrate an afterloaded contraction at room temperature. Shortening from $s = 2{\cdot}61\ \mu$ to $s = 2{\cdot}14\ \mu$; stimulated at 100/sec. Sensitivity and speed figures refer to 1 grid square. 0·96 mm shortening equivalent to 0·26 μ decrease in striation spacing in *A–G*; 0·34 μ in *H*.

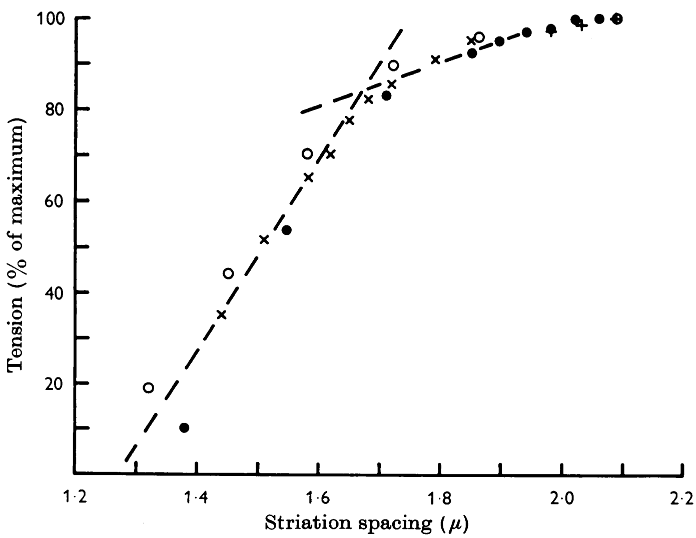

Fig. 5. Tension developed at lengths below the optimum. Open circles, 7. xi. 63, from records reproduced in Fig. 4. Filled circles, 21. x. 63, 5° C. Crosses, 14. xi. 63, 4° C. Plus sign, means from Table 1 (experiments near the optimum length).

Validity of results from afterloaded contractions

The majority of the results presented so far are derived from afterloaded tetani with a shortening stop at the desired length, and it may be questioned how far the results depend on the initial length and on the size of the afterload, which were chosen somewhat arbitrarily. These points have not yet been investigated thoroughly, but the experiments to be described here suggest that effects of these kinds are not important.

Variation of initial length. Figure 6 shows four records from a series of afterloaded contractions in which the initial length was varied while the amount of shortening was adjusted so that the final length, at which the fibre developed tension isometrically, stayed the same (striation spacing 1·95 μ). The maximum tensions produced in these contractions are plotted against initial length in Fig. 7. It will be seen that the tensions developed on shortening from spacings of 2·63, 2·75 and 2·87 μ are identical within experimental error; on shortening from greater lengths the tension drops by a few per cent. The drop of 9% shown for the contraction starting from 3·23 μ is an over-estimate since the shortening took so long that not enough time remained during the tetanus for the maximum tension to be reached (see Fig. 6*C*), and in any case it does not appear large when one recalls that the isometric tension at this length is only about 30% of that at the optimum length.

There is no evidence at present whether the slight decrease of tension in the tetani with shortening from beyond 2·9 μ is related directly to the greater shortening, or to the fact that tension is developed later in the tetanus, or to irregularities in striation spacing that develop during the shortening, or to other unknown factors.

The longest initial striation spacing in the contractions on which Table 1 and Fig. 1 (plateau experiments) are based was 2·6 μ, well below the range where Fig. 7 shows an effect. Figure 3 contains two points (the highest ones in the experiments of 4 December 1964 and 8 December 1964) derived from contractions in which the initial striation spacing was 3·1 μ, at which length Fig. 7 shows a drop of 5%; the effect, if any, is likely to be smaller in the points of Fig. 3 because the amount of shortening was only a quarter as great (0·3 μ change of spacing). In all the other contractions reported in this paper, the initial spacing was below 2·9 μ, the point where Fig. 7 suggests that a fall-off may begin.

Isometric and isotonic contractions. Figure 8 shows records from an experiment designed to test whether isometric and isotonic contractions approach the same curve of tension against length. Figure 8*A* shows a contraction under light load with shortening arrested at a striation spacing of 1·57 μ. The tension developed isometrically at this length was measured

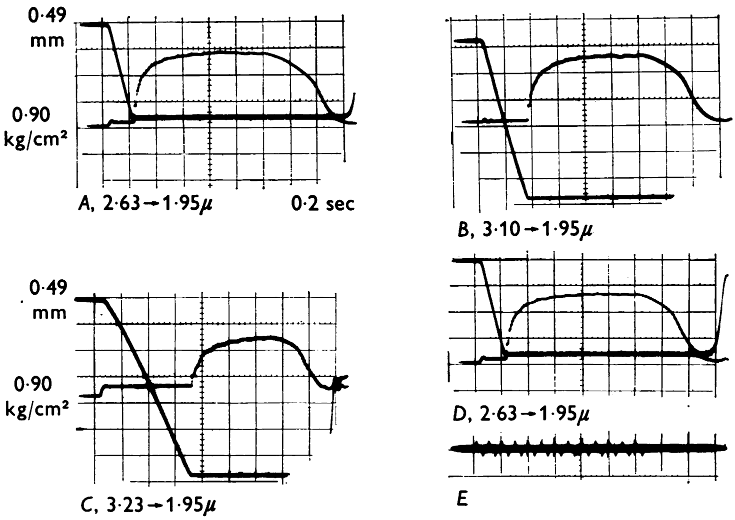

Fig. 6. Development of tension at the same length after shortening under afterload from different lengths. Upper trace, distance between markers (proportional to striation spacing); lower trace, tension. Striation spacing at final length 1·95 μ in all cases; initially 2·63 μ in A, 3·10 μ in B, 3·23 μ in C, 2·63 μ again in D (consecutive tetani in that order). E: stimuli. Experiment of 28. i. 63; temperature 6° C. Sensitivity and speed figures refer to 1 grid square; 0·49 mm shortening is equivalent to 0·194 μ decrease of striation spacing.

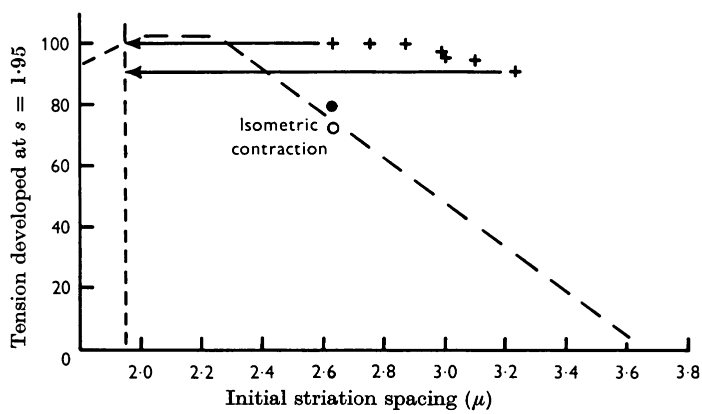

Fig. 7. Results of experiment from which Fig. 6 is taken. Plus sign, tension developed at 1·95 μ, plotted against the striation spacing from which the fibre shortened. Tension expressed as percentage of that developed on shortening from 2·63 μ. The right-hand two points correspond to B and C of Fig. 6. Filled circles, maximum tension, and open circles, tension extrapolated back to start of tetanus, in an isometric servo-controlled tetanus at 2·63 μ. Broken line indicates tetanic tension as function of sarcomere length, from other experiments in this paper.

on the cathode ray tube face and another contraction (Fig. 8*B*) was carried out with the afterload set at this value, and with no shortening stop. Figure 8*C* shows a repeat of *A*. In spite of the long duration of the isotonic contraction, shortening is not over at the end of the record, and the asymptote was found by plotting the slope of the record (speed of shortening) against amount of shortening and extrapolating to zero slope. The results are plotted in Fig. 9, from which it is seen that the isotonic point falls satisfactorily close to the isometric.

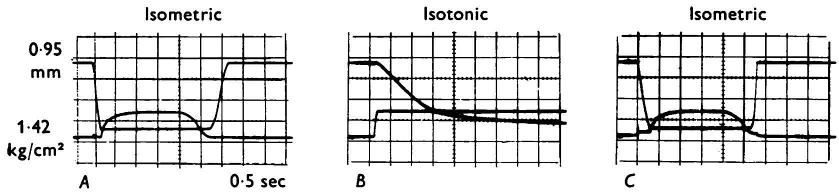

Fig. 8. Comparison of isometric (*A* and *C*) and isotonic (*B*) approaches to steady-state length–tension curve. Experiment of 19. xi. 63; 4° C. Upper trace, shortening; lower trace, tension. Initial striation spacing 2·23 μ in all cases. Sensitivity and speed figures refer to 1 grid square; 0·95 mm shortening is equivalent to 0·21 μ decrease of striation spacing.

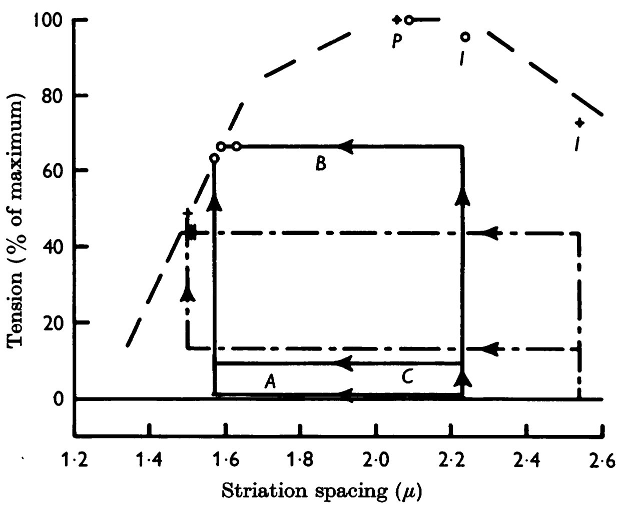

Fig. 9. Isometric and isotonic approaches to length–tension curve. Circles and continuous lines, experiment of 19. xi. 63, including the contractions reproduced in Fig. 8 which are indicated by the letters *A*, *B* and *C*. Crosses, experiment of 14. xi. 63, 4° C. *I*, isometric at initial length of the afterloaded contractions. *P*, afterloaded contractions with shortening arrested within the plateau. Of the two points on each isotonic line, the right-hand indicates the greatest shortening actually recorded and the left-hand indicates the final length extrapolated by the procedure described in the text.

The time constant with which the shortening in an isotonic contraction approaches its asymptote should be inversely proportional to the slope of the length–tension curve at the steady-state point, since the speed of shortening should be proportional to (P_0-P). The slope of our mean curve is about four times as great at lengths below the 'corner' at 1·67 μ as above it; hence the approach to the asymptote is likely to be too slow to obtain satisfactory results above the corner.

Speed of shortening under light load

The force–velocity relation has not yet been systematically investigated over the whole range of lengths, but some preliminary observations will be described here because they are relevant for the interpretation of the effects of change of length. A short account of these experiments was given by A. F. Huxley & Julian (1964).

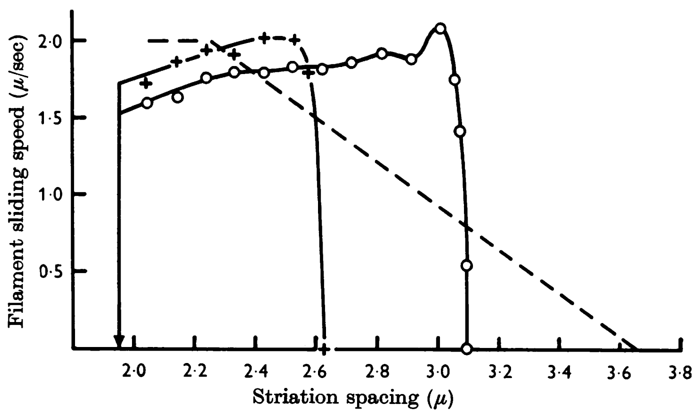

Fig. 10. Speed of shortening under very light load, from records in Fig. 6. Circles: shortening from 3·10 to 1·95 μ striation spacing (Fig. 6*B*). Plus signs: shortening from 2·63 to 1·95 μ striation spacing (mean from *A* and *D*, Fig. 6). Interrupted line: isometric tetanus tension (approximate).

Unloaded shortening above the plateau. Figure 6*B* shows a free-loaded isotonic tetanus in which a fibre shortens from an initial sarcomere length of 3·10 μ until the contraction becomes isometric at 1·95 μ. The length record is nearly straight, showing that the speed of shortening was almost constant. The slope of the record was measured at several points, and the results are plotted against sarcomere length in Fig. 10 (circles), the ordinate being expressed as the relative velocity of thick and thin filaments in each half-sarcomere. The slight overshoot at the start is likely to be due to imperfections in the servo system, since the tension records shows oscillations at the same time, but apart from this the speed of shortening changes little, declining by about 15% whereas the isometric tension increases, over the same range of length, from about 40 to 100% of its maximum value. The isotonic load during this contraction (equal to the resting tension at the initial length) was about 4% of the maximum tetanic tension.

In the same experiment, contractions starting from other lengths were also recorded. The means from two contractions starting from 2·63 μ striation spacing (Figs. 6*A* and *D*) are plotted as the crosses in Fig. 10. It is seen that these values are again nearly constant and only slightly higher than those in the contraction from 3·10 μ.

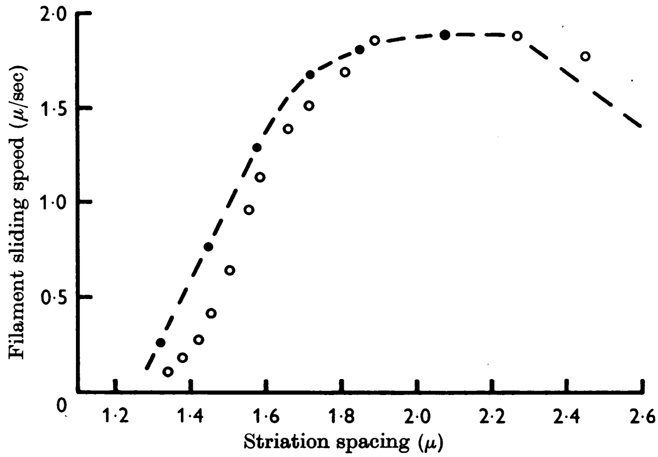

Fig. 11. Speed of shortening (open circles) under light load from 2·57 to 1·32 μ striation spacing. From the contraction shown in Fig. 4*G*. Filled circles and interrupted line: $(P_0 - P)$, scaled to match speed of shortening in the plateau (same experiment).

Shortening below the plateau. Records from an afterloaded contraction with light load (6% of maximum tetanic tension) are reproduced in Fig. 4*G*. The speed of shortening is plotted against striation spacing in Fig. 11. It is nearly constant at first, but begins to drop after the striation spacing reaches 1·9 μ, and this fall becomes very marked after 1·6 or 1·65 μ.

According to Abbott & Wilkie (1953), the speed of shortening in a muscle below its optimum length is given by A. V. Hill's (1938) characteristic equation

$$(P+a)(V+b) = (P_0+a)b, \tag{1}$$

with a and b constant, provided that the isometric tension P_0 is always given the value appropriate to the length at which the speed of shortening V is measured. In an isotonic contraction, P is constant and the equation simplifies to

$$V = \frac{b}{P+a}(P_0 - P) \tag{2}$$

in which the first term is constant. P_0 was measured at a series of lengths in the experiment from which Fig. 11 is constructed; hence $(P_0 - P)$ was found and is plotted in Fig. 11 on a scale which makes it agree with the speed of shortening at a spacing of 2·1 μ. Below 1·9 μ the observed velocities fall progressively below the values of $(P_0 - P)$, showing that the relation suggested by Abbott & Wilkie does not hold; Aubert (1956, p. 230) found a deviation in the same direction. Below about 1·4 μ, the observed velocity is about a third of the value to be expected on the basis of proportionality to $(P_0 - P)$.

Similar results were found in three other experiments except that the fall in velocity from a spacing of 2·0 μ to a spacing of 1·7 μ was relatively less rapid than the fall of $(P_0 - P)$, while in Fig. 11 it is more rapid. All experiments showed the acceleration of the decline below about 1·6–1·7 μ, and the flattening out of the curve near 1·4 μ at values about a third of what would correspond to $(P_0 - P)$.

DISCUSSION

Interpretation of results on the sliding filament theory

In the Results section and in the preceding paper, the variation of tetanus tension with striation spacing was presented separately for the different parts of the range. These results are summarized and brought together in Fig. 12, in which the vertical lines are placed so as to pass through the intersections of the straight parts of the complete length–tension curve. Many features of this curve can be given a simple interpretation on the basis of the sliding filament theory.

The best available values for the dimensions of the contractile structures of frog muscle, for comparison with the critical sarcomere lengths indicated in Fig. 12, are those given by Page & H. E. Huxley (1963) for the lengths of the filaments and by H. E. Huxley (1963) for the distribution of 'bridges' along the thick filaments; using the nomenclature indicated on Fig. 13, their values are: $a = 1·6\,\mu$ (length of thick filaments); $b = 2·05\,\mu$ (length of thin filaments); and $c = 0·15–0·2\,\mu$ (width of region of thick filament with no bridges). The width of the Z line, z, may be taken as 0·05 μ, obtained by measurement of published electron micrographs (Knappeis & Carlsen, 1962; Page & H. E. Huxley, 1963).

There are six stages in the shortening process at which qualitative changes occur in the relations between the thick filaments, the thin filaments and the Z lines. These stages are shown in Fig. 14 together with the values of sarcomere length at which they should occur on the basis of the dimensions given in the preceding paragraph. These sarcomere lengths are indicated in Fig. 12 by the numbered arrows at the top. The agreement between the positions of corner A of the length–tension curve (zero tension) and stage 1 of the sliding process (zero overlap) has been discussed in the preceding paper. Corners B and D evidently coincide well with stages 2 and 5 respectively of the development of overlap. Corner C may be related to stage 3, but corner E does not fit satisfactorily with any of the landmarks of the shortening process, and any effect which stage 4 of shortening may have does not show up in these results.

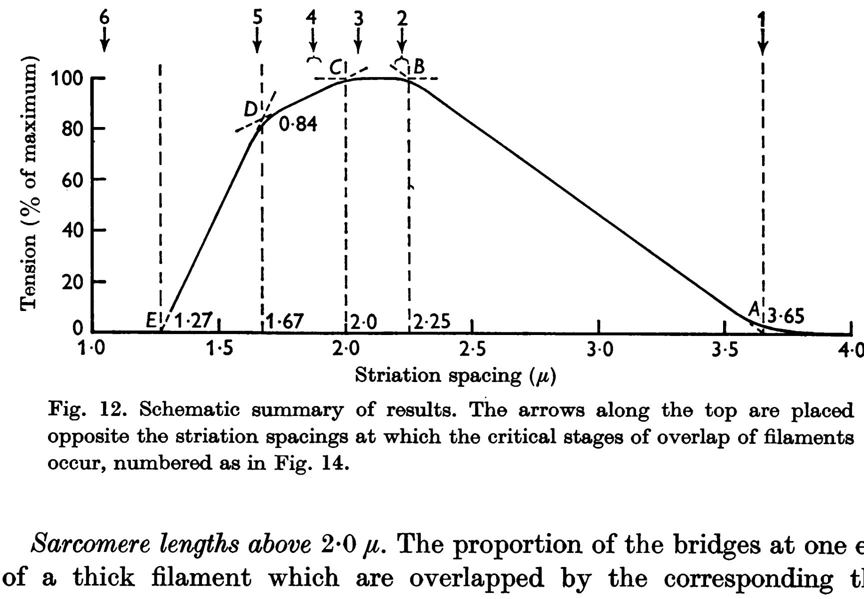

Fig. 12. Schematic summary of results. The arrows along the top are placed opposite the striation spacings at which the critical stages of overlap of filaments occur, numbered as in Fig. 14.

Sarcomere lengths above $2{\cdot}0\,\mu$. The proportion of the bridges at one end of a thick filament which are overlapped by the corresponding thin filaments increases in direct proportion to shortening from stage 1 to stage 2 and then remains constant (assuming a uniform distribution of bridges in the part of the thick filaments where they are present). The agreement of these stages with corners A and B of the experimental length–tension curve thus shows that isometric tension is directly proportional to the number of bridges overlapped, for all spacings above the lower limit of the plateau at $2{\cdot}0\,\mu$ (disregarding for the time being the rounding of the corners of the length–tension curve). This suggests strongly that a fixed amount of relative force between thick and thin filaments is generated at each point where a bridge is overlapped by the thin filament belonging to its own end of the sarcomere, and that the total tension on, say, a thin filament in the I band is the sum of the forces exerted on it at each of the bridges which it overlaps. This is substantially the suggestion of A. F. Huxley & Niedergerke (1954), with the further proposition that the points where the force is generated are the bridges, as was suggested by H. E. Huxley (1957).

The remarkable constancy of the speed of unloaded shortening in this range of lengths (p. 182) also suggests that the only important effect of changing the length is to alter the number of sites acting in parallel. In terms of A. V. Hill's (1938) characteristic equation, a as well as P_0 would be proportional to the number of sites active so that $a = \alpha P_0$ while b

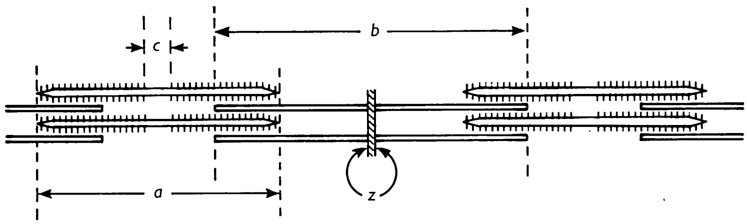

Fig. 13. Schematic diagram of filaments, indicating nomenclature for the relevant dimensions.

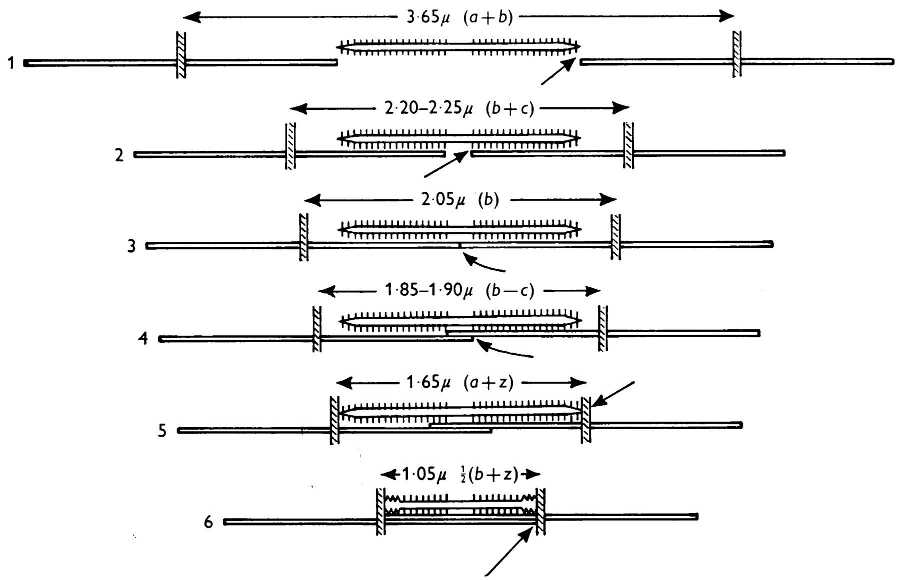

Fig. 14. Critical stages in the increase of overlap between thick and thin filaments as a sarcomere shortens.

would be unchanged. Inspection of eqn. (2) (p. 183) shows that when P is small these assumptions lead to $V = b/\alpha$, which is independent of P_0. Alternatively, as pointed out by A. F. Huxley (1957, p. 292), both the force generated and the internal resistance to shortening are likely to be proportional to the number of sites active, so that the ratio of these quantities, which determines the speed of shortening when the external load is zero, will not alter. It is possible that a genuine viscous resistance to shortening would also be proportional to the length of the overlap zone, so the observation of constant speed of shortening does not help to distinguish between this kind of resistance and a resistance related to the number of sites active.

It has been found by X-ray diffraction that the lateral spacing between filaments varies with stretch in such a way as to keep the lattice volume nearly constant (H. E. Huxley, 1953; Elliott, Lowy & Worthington, 1963). Further, Elliott, Lowy & Millman (1965) have shown that there is only a slight change in lateral spacing when a muscle is stimulated at any of a wide range of sarcomere lengths. They conclude that interaction between actin and myosin filaments can take place over a widely variable distance (200–250 Å centre to centre). The interpretation given here for both the variation of tension and the constancy of speed of shortening implies further that the force and speed parameters of the interaction at any one site are unchanged when the distance between the filaments alters to this extent.

Lower limit of the plateau. In our preliminary notes (Gordon *et al.* 1964; A. F. Huxley, 1963), it was suggested that the drop of tension which begins as the spacing is reduced below about 2·0 μ might be related either to stage 3 or stage 4 of the shortening process, i.e. meeting of thin filaments at the centre of the sarcomere or the beginning of overlap with bridges at the far end of the thick filament, respectively. The more recent series of experiments reported in this paper put the lower limit of the plateau at a slightly longer striation spacing than the earlier series, and therefore make the second of these suggestions rather unlikely as the cause of the initial fall. It may of course contribute to the continued fall beyond 1·9 μ but our experiments provide no evidence on this point.

Another factor which may be relevant is that the slack length of the fibres is at about 2·1 μ striation spacing. Ramsey & Street (1940) showed that a fibre extends itself to the slack length on relaxation after shortening below it, and this means that below this length there must exist a force tending to extend the fibre and therefore presumably subtracting from whatever tension is developed by the filaments during contraction. There is no basis at present for estimating the size of any such force. It is possible also that the slack length is partly or wholly determined by the collision of thin filaments at the middle of the *A* band, so these two factors may not really be distinct.

Short lengths. The corner *D* where the slope of the length–tension diagram increases is almost exactly at the striation spacing corresponding to stage 5, i.e. collision of ends of thick filaments with the *Z* lines. This would be expected to cause added resistance to shortening, and it is also likely that crumpling or folding of the thick filaments would reduce the number of bridges capable of generating tension.

Forces opposing shortening

Of the factors suggested in the last few paragraphs to account for the decline of tension with decrease of length, some would act by reducing the intrinsic force generated by the contractile system while others would act by producing an opposing force. Aubert (1956, p. 194) suggested that factors of this second kind were important below the optimum length, because he found that the maintenance heat rate (and particularly its 'stable fraction' which may be regarded as a measure of relevant contractile activity), falls off less rapidly than the tension exerted externally, while above the optimum length he found a direct proportionality. The large fall in speed of shortening under light load (Fig. 11) would also be explained by the existence of large forces opposing shortening, as may be seen from the following argument.

If there is an internal force P_r resisting shortening, then both the isometric tension generated internally and the effective resistance to shortening are increased by this amount above the externally-recorded values and eqn. (2) becomes

$$V = b\,\frac{\{(P_0+P_r)-(P+P_r)\}}{P+P_r+a},$$

or

$$V = \frac{b(P_0-P)}{P+P_r+a}. \tag{3}$$

At very short lengths, V was about one third of the values expected on equation (2) with constant a; hence, the denominator of (3) must be three times that of (2), or, if a is still assumed constant,

$$P_r = 2(P+a).$$

Taking $a = \frac{1}{4}P_{max}$, and with $P = 0{\cdot}06\,P_{max}$, this gives $P_r = 0{\cdot}62\,P_{max}$, suggesting that more than half the drop of tetanic tension represents an internal force opposing shortening rather than a decrease of intrinsic force of contraction; an even larger fraction would have been found if a had been assumed to fall. It is not worth pursuing this argument in a more quantitative fashion as the results would depend heavily on assumptions concerning the dependence of the internal resistance on the speed of shortening.

Another factor which may produce resistance to shortening is the deformation of the sarcolemma accompanying the increase of diameter of the fibre. If this causes a rise of pressure within the fibre, the externally recorded tension will be reduced by the force exerted by this pressure on each end of the fibre, i.e. the product of pressure and cross-sectional area. Any longitudinal component of tension in the sarcolemma would add to the recorded tension, but this is unlikely to be large because of the shortening of the fibre and the decrease of total surface area. The following calculation suggests that the pressure effect may be considerable. Sato (1954) found that isolated fibres tetanized under light load decreased in volume by 10–30 % when shortened by 40–75 % of initial length. If this is an extrusion of water without solutes, the internal osmotic pressure must be raised and the hydrostatic pressure must be at least equal to the excess of internal over external osmotic pressure. If the water content of the resting fibre is 0·7 g/ml., even the 10 % decrease of volume would mean a decrease of internal water from 0·7 to 0·6 g/ml. of initial volume. The internal osmotic pressure would therefore rise in the ratio 0·7/0·6, and since the osmotic pressure of Ringer's solution is about 6 atm (= 6 kg/cm^2) the increase would be 1 kg/cm^2, which is about a third of the peak tetanic tension.

Effect of variability of striation spacing

Even if the 'corners' of the length–tension curve were ideally quite sharp, they would be rounded in any real experiment because the striation spacing is not completely uniform. Some idea of the variability is given in Figs. 10 and 11 of the preceding paper; measurements at twenty-one places on each photograph of the fibre of 28 August 1963 (from which Fig. 10 is taken; mean spacing 3·90 μ) gave standard deviations of (*a*) 0·036 μ between measurements on a single frame, and (*b*) 0·039 μ between the means of the seven photographs taken within the stretch between the markers. These figures give an overall standard deviation (*c*) of 0·053 μ. The less highly stretched fibres used in the present paper gave rather lower figures, the corresponding values for (*a*), (*b*) and (*c*) being respectively 0·036, 0·019 and 0·041 μ for the three fibres of Fig. 5 (measured at a mean spacing of 2·58 μ), and 0·027, 0·022 and 0·035 μ for the five fibres of Table 1 (experiments at the plateau; measured at a mean spacing of 2·35 μ). An appreciable part of the variability within a single photograph must be due to the 'vernier formations', a few of which were visible in most frames.

The order of magnitude of the rounding due to this variability can be found by calculating the mean of the tensions appropriate to the different striation spacings present. If the spacing is distributed normally with the mean exactly at the position of the 'corner', and the change of slope of the length–tension relation at the corner is m, then the mean tension deviates from the ideal value by

$$(m/\sigma\sqrt{2\pi})\int_0^\infty x\exp(-x^2/2\sigma^2)\mathrm{d}x$$

which is $m\sigma/\sqrt{2\pi}$.

The change in slope at the upper end of the plateau is from zero to a decline at the rate of P_0 in (3·65–2·25) or 1·4 μ, i.e. $m = -P_{max}/1{\cdot}4$ tension units per micron. Taking $\sigma = 0{\cdot}035\ \mu$ (overall value for the fibres used in the plateau experiments), $m\sigma\sqrt{2\pi}$ is $-0{\cdot}01\ P_{max}$, i.e. the rounding due to variability of striation spacing should bring the curve below the ideal corner by 1 % of the maximum tension. This is of the same order as the deviation seen in Fig. 1.

This calculation is appropriate for the effect of variation between the sarcomere lengths in different fibrils, whose tensions can be assumed to add. For variability along one fibril, it is the length changes which are additive, and these must add up to zero in an isometric contraction. In this case, a calculation of the effect is complicated by the asymmetry between lengthening and shortening (Katz, 1939; Aubert, 1956, p. 218); according to Katz, the speed of lengthening for a given increase of load above the isometric value is a fraction (β, about $\frac{1}{6}$) of the speed of shortening for an equal decrease of load. We have not calculated the amount of rounding on this basis, but in the following paragraph we estimate roughly the size of another effect which is introduced by this rectifying type of action, namely that the width of the plateau is exaggerated.

In a part of the length–tension diagram with constant slope m, the actual tension P exerted isometrically exceeds by an amount $\triangle P$ the true value which corresponds to the mean sarcomere length. $\triangle P$ can be calculated by numerical solution of the equation which represents the condition that the total rate of shortening in sarcomeres whose true isometric tension exceeds P is equal to the total rate of lengthening in sarcomeres whose true isometric tension is less than P. The result is that

$$\triangle P = Km\sigma,$$

where K is a function of β; hence, the length–tension line is displaced by a distance $K\sigma$ parallel to the sarcomere–length axis. For $\beta = \frac{1}{6}$, K is about 0·7, so that a standard deviation of 0·035 μ in striation spacing would displace the line by 0·025 μ. The corners B and C at the ends of the plateau would thus each be displaced outwards by this amount, so that the true value of the separation between them may be only 0·2 μ instead of the 0·25 μ which was found to fit the experimental observations. This calculation may well over-estimate the effect, since any transverse rigidity in the fibre (e.g. between the two sides of a vernier formation) would reduce the length changes.

Agreement with earlier results

The good agreement of our results with those of Ramsey & Street (1940) was emphasized in fig. 1 of our preliminary communication (Gordon *et al.* 1964) in which a line summarizing our results was superimposed on Ramsey & Street's fig. 5. Figure 12 of the present paper differs only slightly from the line used there, and the differences are in the direction to improve the agreement with Ramsey & Street's points. The higher tensions found by Ramsey & Street both at (*a*) long and (*b*) very short striation spacings are almost certainly explained as follows:

(*a*) Ramsey & Street plotted maximum tensions, which included the slow rise due to shortening of the ends of the fibres where the striation spacing is less than in the middle part of the fibre.

(*b*) The substantial tensions recorded by Ramsey & Street at 30–60 % of their standard length were obtained only when the shortening was no longer fully reversible, the fibre having entered the 'delta state'. We avoided the conditions shown by Ramsey & Street to produce the 'delta state', i.e. repeated tetani of long duration with free shortening.

Guld & Sten-Knudsen (1960) found that the isometric twitch tension declined linearly with increase of striation spacing from 2·3 to 3·5 μ, along a line which extrapolated to zero tension at 3·57 μ. The difference between this and our mean line over the same range for tetanic tension (extrapolated back to the start of the tetanus) is probably within the possibilities of experimental error, suggesting that the twitch–tetanus ratio does not vary much within this range.

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